1528 Issued October 20, 1913.

PORTO RICO AGRICULTURAL EXPERIMENT STATION,

D. W. MAY, Special Agent in Charge.

Mayaguez, P. R.

Bulletin No. 13.

STUDIES ON ACID SOILS OF PORTO RICO.

BY

OSCAR LOEW,
PHYSIOLOGIST.

UNDER THE SUPERVISION OF

OFFICE OF EXPERIMENT STATIONS,

U. S. DEPARTMENT OF AGRICULTURE.

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PORTO RICO AGRICULTURAL EXPERIMENT STATION.

[Under the supervision of A. C. True, Director of the Office of Experiment Stations, United States Department of Agriculture.]

Walter H. Evans, *Chief of Division of Insular Stations, Office of Experiment Stations.*

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LETTER OF TRANSMITTAL.

Porto Rico Agricultural Experiment Station,
Mayaguez, P. R., April 4, 1913.

Sir: I have the honor to transmit herewith a manuscript by Dr. Oscar Loew on "Studies on Acid Soils of Porto Rico." On account of the wide extent of such soils in this island and perhaps in the tropical world and their probable influence in limiting plant growth a study of the causes of acidity and methods of amelioration should prove of value. The investigations reported herewith furnish interesting information regarding the organisms which inhabit tropical soils and the relation they bear to agricultural production in such regions.

I recommend that this manuscript be published as Bulletin 13 of this station.

Respectfully,
D. W. May,
Special Agent in Charge.

Dr. A. C. True,
Director Office of Experiment Stations,
U. S. Department of Agriculture, Washington, D. C.

Recommended for publication.
A. C. True, *Director.*

Publication authorized.
D. F. Houston,
Secretary of Agriculture.

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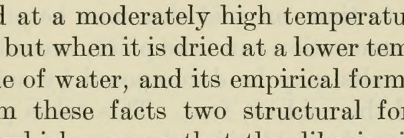
INTRODUCTION.

Acid soils occur much more frequently than has been supposed. In the United States they are widely distributed, and have been studied especially in Illinois, New Jersey, and Rhode Island. It is important to distinguish between soil acidity due to humic acid and that due to mineral compounds. To the latter class belong some of the clay soils of Porto Rico. The formation of this acid clay seems to be especially favored by heavy rains and a high summer temperature, whereby the carbonate of lime formed by disintegration of rock particles is gradually dissolved and leached out and finally even the lime combined with clay is removed. Pure clay behaves like an acid. In neutral clays this acid is neutralized by bases, such as potash, soda, lime, magnesia, or ferric oxid. Only acid clay soils will be considered here.

Pure clay dried at a moderately high temperature has the composition $H_2Al_2Si_2O_8$, but when it is dried at a lower temperature it retains one more molecule of water, and its empirical formula corresponds to $H_4Al_2Si_2O_9$. From these facts two structural formulas have been inferred, both of which assume that the dibasic silicic acid exists in clay and further that the two molecules of aluminum hydroxid present unite to form an anhydrid. But it would be much more in accord with the chemical behavior of clay if orthosilicic acid $Si(OH)_4$ were assumed to be present in clay as has been done in the case of various other silicates. It is also more probable that the silicic acid is in anhydrid form than that the alumina is in this form. A formula based on such an assumption would furnish a natural explanation of the great resistance of clay to even strong mineral acids, as aluminum is held by two affinities to silicic acid.

Zulkowski [1] has suggested the formula,

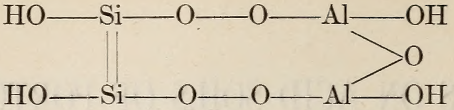
(a)



(b)

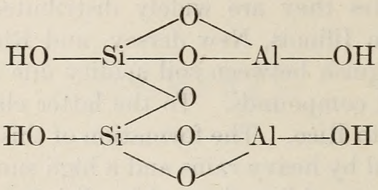
[1] Zulkowski assumes the existence of a kaolinic acid also (Chem. Indus. [Berlin], 22 (1899), p. 280).

while Pukall[1] supposes the formula,



would correspond better to the chemical behavior of clay, but this seems rather improbable.

The following formula would be more in accord with the chemical behavior of clay:

(a)  (b)

This acid clay may be named argillic acid. By neutralization of the acid hydroxyls at (a) the acid clay would become neutral. The absorption of phosphoric acid can be explained by the basic hydroxyls at (b). By prolonged treatment of neutral clay, i. e., salts of argillic acid, with large quantities of water, charged with carbon dioxid, renewed from time to time, an acid clay may be produced. On the other hand, the reverse action can take place, according to the law of mass, i. e., neutral salts may be decomposed by acid clay, the base being absorbed and the acid set free. This takes place to a certain extent even with potassium chlorid and ammonium sulphate. Hence, acid soils, fertilized with neutral salts, will often produce worse results than when not fertilized at all. In this type of soil potassium chlorid should be replaced by potassium carbonate, and ammonium sulphate[2] by sodium nitrate. The best proceeding, however, will be to apply at least enough lime to destroy the acidity and then ammonium sulphate may produce even better results than sodium nitrate.

Since the reaction with litmus paper is not always very decisive, several qualitative tests for acid soils have been worked out. One depends upon the use of a mixture of potassium iodid with potassium iodate plus starch paste. Acid soils liberate the acids from these salts and they in turn act upon each other and produce free iodin, as indicated by the blue coloration of the starch paste. The second method uses a mixture of potassium nitrate, potassium iodid, and starch paste, from which acid soils also liberate iodin. This test is made as follows:[3] Five grams of soil is moistened with a 1 per

[1] Ber. Deut. Chem. Gesell., 43 (1910), p. 2078.

[2] Ammonium sulphate applied continuously on soils poor in lime can hasten the loss of lime by leaching. Such cases have been observed at Rothamsted and by the Rhode Island Station.

[3] This is Daikuhara's modification of the writer's test.

cent solution of potassium nitrate to form a thin paste, which is transferred to a small flask. Then 2 grams of soil is placed on the surface of this paste. A strip of paper, moistened with freshly prepared and slightly acidulated starch paste containing potassium iodid, is hung in the flask, which is closed with a stopper. Only acid soils can liberate nitrous acid from potassium nitrate, and this, on gently warming, will volatilize and set iodin free in the reagent paper, causing a blue coloration of the starch.

From the degree of acidity the lime necessary for neutralization can be calculated. It can be determined approximately by grinding the air-dry clay soil[1] very fine and digesting 50 grams with 200 cubic centimeters of a neutral 1 per cent solution of sodium or potassium acetate at room temperature, with frequent shaking, for 24 hours. The acetic acid is more easily set free than either hydrochloric or sulphuric acid and is titrated in 100 cubic centimeters of the filtrate.

A number of soils were tested in this way, and from the amount of sodium hydroxid required for neutralization of 100 cubic centimeters of the filtrate, the amount of sodium hydroxid required for 1 kilogram of soil was calculated as shown in the following table:

Relative acidity of some Porto Rican soils.

Soil.	NaOH to neutralize 100 cubic centimeters filtrate.	NaOH to neutralize 1 kilogram soil.
	Grams.	*Grams.*
Hormigueros, cane plantation	0.004942	0.1977
Jagua coffee plantation, stiff clay soil	.026317	1.0527
Sandy soil from the coast line	.0	.0
Surface soil from fields west of Bayamon	.017300	.6919
Subsoil from fields west of Bayamon	.039790	1.5913
Anasco, cane plantation	.008649	.3360
Same soil, limed at the rate of 4,000 pounds per acre	.002471	.0988
Rio Grande district:		
No. 1, loamy soil	.006425	.2570
No. 2, sandy soil	.018534	.7413
No. 3, sandy soil	.0111204	.4448
No. 4, sandy soil	.0111204	.4448
No. 5, sandy soil	.008278	.3311
Santa Catalina, loamy soil	.015074	.6029

As already indicated, the acid soils of Porto Rico owe their acidity not to humic acid but to an acid clay. These soils, especially the red clay soils, often contain no trace of humus, and such acid loam soils as contain a little humus have generally an acid subsoil without humus. The acid red clays are generally very poor in organic matter, except in the vicinity of decaying roots.

Soils combining all optimal soil conditions as regards chemical, physical, and biological factors are rare in Porto Rico. Still, if certain faults do not reach extremes, vegetation thrives well, especially in

[1] In the case of coarse soils only the fine earth should be taken for the test.

parts of the island where the rains are sufficient. Certain plants, as the banana, grow with wonderful luxuriance even on very stiff and acid clay soils,[1] as do the orange, pomarosa, mango, and guava. Evidently certain plants adapt themselves to stiffness and acidity of soils in the tropical climate to a degree which in northern countries would be considered well-nigh impossible.[2] Soils in the Tropics can even develop a rich vegetation when the amount of mineral nutrients falls considerably below the minimum required in northern countries, as has been recognized by various observers in the Sunda Islands.

ACID SOILS OF PORTO RICO.

If a "tired" soil be defined as one which, notwithstanding the ample application of fertilizers, does not produce a satisfactory harvest, then the following causes of "tired" soils can be distinguished: (1) Excessive acidity or alkalinity, (2) deficient aeration due to poor physical condition in clay soils or to abnormally high bacterial content, (3) abnormally large numbers of parasitic organisms, and (4) of injurious animal organisms not of a strictly parasitic nature.

The remedy to be applied can only be determined after careful examination of the soil in question. The so-called "soil disinfectants" are often applied with excellent results, but the increase of yield is often only temporary as their effect tends to disappear in two or three years. Exceptions occur, however, when parasitic organisms, as, for example, nematodes or bacteria, causing wilt and other diseases in certain crops, have been destroyed by the disinfection. Sometimes a judicious rotation of crops may prove as beneficial as disinfection.

Apparently "tired" soils are not infrequently encountered in Porto Rico, and some cases have been described by the writer.[3] In the following account investigations on certain acid soils are reported.

SOIL FROM HORMIGUEROS.

On extensive tracts of land under cane at Hormigueros the yields have decreased for a series of years, amounting at present to only about 14 tons of cane per acre as compared with 40 to 50 tons on similar soils in other parts of the island. In 1911 the chemist of the station started experiments on these fields, and preliminary examinations

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[1] The daily rains and thunderstorms seem to furnish more available nitrogen in Porto Rico than in northern climates.

[2] As regards plant development in acid soils, there is a great difference in the sensitiveness of the roots. Maxwell (Jour. Amer. Chem. Soc., 20 (1898), No. 2, pp. 103–107) found that Cruciferæ, alfalfa, wheat, and barley, are more easily injured than bean, lupine, vetch, and maize; millet was found most resistant. Buckwheat, oats, and potatoes succeed well on acid muck soils, where barley and wheat fail. Some varieties of certain species may do better on acid soils than other varieties of the same species. F. V. Coville (U. S. Dept. Agr., Bur. Plant Indus. Bul. 193) reports that the blueberry is unable to grow in neutral soils, but thrives best in acid peat soils. See also F. W. Card, Rhode Island Sta. Bul. 91 (March, 1903).

[3] Porto Rico Sta. Circ. 12.

were made in regard to the soil conditions of some of the principal experimental plats. Samples were carefully taken to depths of 6 and 18 inches. It was noticed that even at a depth of 18 inches a moderate degree of porosity exists in this rather stiff loam soil, due to repeated deep plowing.

The first test was that for ferrous oxid, which may be formed as a result of butyric fermentation in the roots of the sugar cane, which produces not only butyric acid but also carbon dioxid and hydrogen, the latter in a nascent state, reducing ferric oxid to ferrous oxid. Such reduction can be easily observed when a ferric salt is added to a saccharine liquid undergoing butyric fermentation. The presence of ferrous oxid in compact soils would be deleterious in so far as it would contribute to the suffocation of roots by the absorption of the oxygen in the small amount of air that penetrates the soil. All the samples examined soon after collecting failed to yield ferrous oxid on extraction at room temperature with 1 to 2 per cent sulphuric acid. The filtrate was tested with potassium ferricyanid and in no case gave the blue reaction for ferrous oxid. From this it can be inferred that the aeration of the soil was sufficient.

The second test was that for calcium carbonate in the plat limed at the rate of 4 tons per acre. This heavily limed soil was found to contain no calcium carbonate. The lime was just sufficient to neutralize the acid reaction of the soil. For successful cane culture still more lime, say a total of 6 to 8 tons per acre, may be necessary, the lime factor for cane being at the same time improved.[1] In liming it should always be borne in mind that a large amount of lime is annually lost through leaching out by rains. At the Rothamsted Experiment Station the annual loss of calcium carbonate in the drainage was found to be 500 to 1,000 pounds per acre. The use of ammonium sulphate increased the loss, while sodium nitrate diminished it. This is particularly important in case of soils poor in lime. On the other hand, the loss of nitrate by leaching and denitrification should be taken into account.

The next question was whether heavy liming affects the amount of the butyric ferment. These bacilli occur in every soil even at considerable depths, but their number increases with the presence of decaying roots rich in starch or sugar. These materials easily undergo butyric fermentation and thus the butyric bacillus, although a nitrogen gatherer, can do more harm than good under certain conditions, since the butyric and acetic acids it produces increase the acidity of the soil. As Azotobacter does not produce fermentation, it is a much more valuable nitrogen gatherer than the butyric bacillus.

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[1] According to determinations of the assistant chemist of the station, this surface soil contains 0.61 per cent CaO and 1.26 per cent MgO.

6748°—13——2

Two grams of soil taken at a depth of 6 inches on the limed plat and the unlimed check plat, with 40 cubic centimeters of sterile nitrogen-free culture solution [1] colored yellow by methyl orange, were placed in an apparatus which permitted the measuring of the gas produced by fermentation (carbon dioxid and hydrogen) at a temperature of 24 to 33° C. Figure 1 shows a convenient form of apparatus for this purpose. Two grams of a sandy soil from the coast at Pena Cortada,[2] taken at the same depth, served for comparison. The results are given in the table following.

Evolution of gas from soils through the action of soil organisms.

	Volume of gas developed in—				Remarks.
	2 days.	3 days.	4 days.	5 days.	
Loam soil of Hormigueros:	*Cc.*	*Cc.*	*Cc.*	*Cc.*	
Plat limed at rate of 4 tons per acre.	2.5	10.2	14.3	18.0	Solution decolorized.
Check plat	2.4	10.0	14.0	17.6	Do.
Sandy soil from the coast (unmanured soil of a coconut plantation)	0.0	3.0	6.7	11.6	Solution not decolorized.

It will be seen that the butyric fermentation was about equal in both the limed and unlimed soil, from which it is inferred that the number of butyric bacilli was not materially affected by liming. Further, there is considerably less butyric fermentation in the sandy soil from the coast than in the loam soils of Hormigueros. Another interesting difference is that methyl orange was not decolorized by the fermentation in case of the sandy soil, but this was very quickly accomplished in case of the loam soil.

It must be borne in mind that after the fermentable material, such as sugar and starch, is destroyed the butyric bacilli, before dying, form spores which may remain for years in an inactive but living condition and awake to activity again and germinate as soon as suitable organic nutrients reach them. Some fragments, corresponding to 0.70 gram in the dry state, of badly decayed banana root,[3] consisting mainly of fiber and the cuticularized layer of the bark, and similar fragments of cane root, corresponding to 0.52 gram dry matter, were placed in the apparatus for measuring the intensity of butyric fermentation. The fermentation commenced about 12 hours afterwards and became very active as shown in the table following.

[1] This solution contains glucose 1 per cent, dipotassium phosphate 0.2 per cent, magnesium sulphate 0.02 per cent. The latter was added in sterilized solution after sterilization of the glucose solution.

[2] This sandy soil is very poor in calcium carbonate, except in the immediate vicinity of the coast line.

[3] The dying roots are at first attacked by fungi, which by their enzyms open the way for the butyric microbes. The organic acids the latter produce by fermentation are gradually destroyed by mold fungi and aerobic microbes. The soft parts of the cortex disappear first and later the hardest wood fibers and the cuticularized corky layer of the bark disappear through the action of mycelium of fungi.

Gaseous fermentation of cane and banana roots.

Material.	Gas developed in—			
	12 hours.	36 hours.	60 hours.	80 hours.
	Cc.	*Cc.*	*Cc.*	*Cc.*
0.52 gram cane root	2.6	8.3	14.7	19.1
0.70 gram banana root	2.1	7.2	12.4	16.9

These figures leave no doubt that such dead root fragments are covered by an immense number of spores of the butyric bacillus.[1]

The next question to be considered was that of the presence and relative amount of the nitrogen gatherer, Azotobacter. The multiplying cells of Azotobacter in the test flasks soon form fragments of a film on the surface, which gradually unite and finally form a continuous film. Since such tests also show the presence of protozoa, which grow and multiply rapidly in these test liquids, attention was also paid to the relative amount of these which developed in a given time.

Five grams of soil of differently treated plats was placed with 2 grams of sterilized calcium carbonate in 30 cubic centimeters of a sterilized nitrogen-free culture solution containing 1 per cent glucose, 0.2 per cent monopotassium phosphate, and 0.02 per cent magnesium sulphate. This mixture was kept in conical flasks of 200 cubic centimeters capacity at 24 to 33° C. The results of the microscopic examination, made after four days,[2] are shown in the table following.

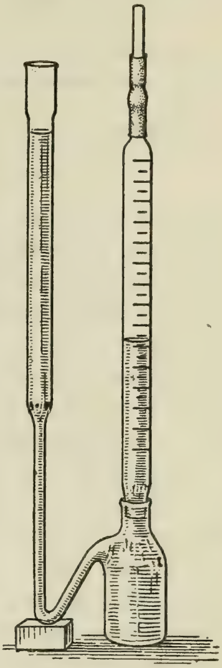

Fig. 1.—Apparatus for comparing the butyric fermentation of different soils. The soil and calcium carbonate are placed in the flask, the graduated tube with India rubber tube is attached, and the apparatus is entirely filled with a nitrogen-free glucose culture solution by the funnel tube. After closing the graduated tube with a glass rod, the funnel tube is emptied. The gas produced collects in the graduated tube and its volume is measured.

[1] According to reliable calculations a hundred million of these or similar microbes on an average occupy a volume of only 0.15 cubic millimeter.

[2] This examination of various soils was made as soon as a film of Azotobacter in one of the flasks appeared. Sometimes a second examination was made three to four days later.

Development of films of Azotobacter and presence of protozoa.

Plat No.	Treatment of plat.	Development after 3 days.	
		Samples from depth of 6 inches.	Samples from depth of 18 inches.
3	Check, plowed 6 times	Moderate film in course of development. Large protozoa, as *Colpoda cucullus.*	Small fragments of a film; few small protozoa.
4	4 tons of lime per acre; plowed 6 times.	Thick film luxuriantly developed, numerous large protozoa.	Film fragments; small protozoa, large rare.
5	Stable manure; plowed 6 times.	Moderate film; numerous protozoa.	No film yet but Azotobacter present. Small protozoa in moderate amount.
19	Check, plowed twice	Film fragments; few protozoa	Film fragments; few small protozoa.
30	Treated with carbon bisulphid.	Luxuriant thick film; many large protozoa.	No film yet but Azotobacter cells present. Small protozoa moderately abundant.
31	Stable manure	Film fragments; numerous protozoa.	Do.

When a soil is very rich in butyric bacilli, it is desirable to replace the glucose by sodium acetate with addition of 0.1 per cent calcium chlorid for the Azotobacter cultures. Thus the disturbing development of butyric bacilli is prevented and a fine film of Azotobacter will develop after three to six days at 24 to 30° C.

It is often stated that Azotobacter and protozoa occur only in the upper strata of the soil of cultivated fields. It therefore appears to be of considerable interest to report that these organisms were encountered in this loam soil at a depth of 18 inches. This is perhaps explained by previous deep plowing to the depth of about 20 inches whereby the soil was considerably loosened and the upper strata mixed with the lower. Cultures of soil from the limed plat and the plat treated with carbon bisulphid yielded luxuriant films of Azotobacter in three days at 24 to 33° C., while soils from the other plats were just beginning to form such films at the end of that time.

A comparative test of the denitrifying power was carried out and the nitrogen set free from sodium nitrate in different soils was measured by means of the same apparatus that served for the study of butyric fermentation. (See fig. 1, p. 11.) Two grams of soil was used for the test.[1] The solution used had the following composition: Ethyl alcohol 2 per cent, sodium nitrate 0.2 per cent, dipotassium phosphate 0.2 per cent,[2] and magnesium sulphate 0.02 per cent.

[1] The first step in the denitrification process is the formation of nitrite and the second the development of free nitrogen from this nitrite. These two phases are represented by the following equation when alcohol serves as the organic food:

$$\text{I. } \underbrace{C_2H_6O}_{\text{Alcohol.}} + \underbrace{6NO_3Na}_{\text{Sodium nitrate.}} = 2CO_2 + 3H_2O + \underbrace{6NO_2Na}_{\text{Sodium nitrite.}}$$

$$\text{II. } C_2H_6O + 4NO_2Na = 2CO_3Na_2 + 3H_2O + 4N.$$

The carbon dioxid indicated in the first equation is not set free but forms with the sodium carbonate of the second equation sodium bicarbonate.

[2] The dipotassium phosphate must show a weak *alkaline* reaction.

After standing six days at 24 to 33° C., the soil of the check plat had developed 9.2 cubic centimeters nitrogen, while the same soil taken at a depth of 18 inches gave no trace of nitrogen gas and the sandy soil of the coast only 0.2 cubic centimeter. This shows that denitrifying organisms are only present in moderate amounts in surface soil and are absent at a depth of 18 inches in the loam soil.

According to the investigation of the plant pathologist of the station, the cane fields at Hormigueros are extensively infected with the fungus Marasmius, which is very injurious to cane. Another fungus, principally attacking the leaves, occurs very frequently. Very probably a thorough soil treatment with carbolineum might be effective in destroying the injurious fungi in the soil, but considering the large areas in question this would involve a very considerable expense. A much better plan would be the carrying out of the proposal made some years ago by the experiment station, namely, that the growth of cane be replaced by that of beans or other nongramineous crops for one or two years on the infected areas.

In connection with this treatment the soil should be limed at the rate of about 6 to 8 tons of calcium carbonate per acre. Liming at the rate of 4 tons is just about sufficient to neutralize the acidity of the soil; hence an increased dose should be applied, not only to provide a more favorable lime ratio for the cane but also to furnish still more favorable conditions for the nitrogen-gathering Azotobacter, which grows best in soils that contain some calcium carbonate. In addition a loosening of the rather stiff loam soil would be produced.[1] To what a great extent acid cane soils are improved by liming was demonstrated some years ago at the station, where when no lime was applied a yield of 44 tons of cane per acre was obtained; when 500 pounds of lime per acre was applied the yield was 50 tons, and an application of 3,000 pounds gave 69 tons per acre.[2]

SOIL FROM ANASCO.

There is a moderately stiff loam soil a short distance north of Anasco which shows an acid reaction. On this soil there is produced about 40 tons of cane per acre, considerably more than at Hormigueros, but still much less than on the loose soils of Hawaii.

The chemist of the station began some fertilizer experiments in 1912 on a cane plantation near Anasco, and the action of lime on the development of Azotobacter, the butyric ferment, and protozoa in the soil is not without interest. It was found that an application of 4,000 pounds of lime per acre did not neutralize the acidity of the soil, and the soil gave no effervescence with hydrochloric acid.

[1] Calcium carbonate accelerates the formation of humus from plant residues in the soil, as Hilgard has pointed out (Agr. Science, 6 (1892), p. 28; Soils, New York and London, 1910, p. 380). Calcium carbonate in moderate amount has been declared to act as a regulator of chemical, biological, and physical processes.

[2] Porto Rico Sta. Rpt. 1910, p. 24.

The limed plat contained more of the butyric ferment than the unlimed, the fermentation in 2 grams of soil yielding in 80 hours with unlimed soil 8.2 cubic centimeters of gas and with the limed soil 10.8 cubic centimeters. This difference may find its explanation in the dissolving action of lime on organic compounds serving as sources of carbon for the butyric bacillus. Since the butyric fermentation was about the same on the limed and the unlimed Hormigueros soil, there was apparently sufficient soluble organic matter present in this case.

Acidity was more marked with the Anasco soil than with similar Hormigueros soil. Fifty grams of soil from the check plat digested for 24 hours with 200 cubic centimeters of a 1 per cent sodium acetate solution gave a filtrate of which 100 cubic centimeters required 0.7 cubic centimeter of soda solution [1] for neutralization, while with the soil of the limed plat only 0.2 cubic centimeter was necessary; the unlimed soil at Hormigueros required 0.46 cubic centimeter.

No Azotobacter was found at a depth of 18 inches. At a depth of 6 inches it was present, and the soil from the limed plat yielded a very luxuriant growth of Azotobacter in four days, while from the unlimed plat only a weak growth was observed in the same time.

A comparison of the soils from Anasco [2] and Hormigueros leads to the inference that the greater yield on the former can hardly be due to better chemical or physical conditions and must be caused by biological factors. The former soil is probably less infected by injurious fungi than the latter. The lime-magnesia ratio is just as unfavorable for cane in the former soil as in the latter, namely, 0.65 per cent lime to 1.18 per cent magnesia, while in Hormigueros soil it was found to be 0.61 per cent lime to 1.26 per cent magnesia. For a favorable development of cane the amount of lime should be at least double that of magnesia.

On such soils the nitrogen might best be applied in form of calcium nitrate, since by this means both nitrogen and lime are added at the same time. Water-soluble compounds, especially when applied in two to three fractions as top dressing, have a much more powerful action than those soluble only in carbon dioxid or other acids.

Among the protozoa observed the leading form was *Colpoda cucullus*. Small Flagellata were found at a depth of 6 inches, but they were absent at a depth of 18 inches.

SOIL FROM THE COLLEGE PLANTATION AND THE EXPERIMENT STATION.

The fact that coffee culture fails in some parts of the college plantation near Mayaguez while it succeeds very well in the immediate vicinity gave rise to the supposition that the soil is "sick" or "tired"

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[1] 1 cc.=0.012356 gm. NaOH.

[2] Plowed once to a depth of 18 inches and once to a depth of 6 inches.

in certain spots. The soil is a very stiff red clay, acid in reaction, and occupies a great area, extending from the orchard hill of the experiment station far up into the mountains at the Aurelia plantation. This clay soil stratum is of considerable thickness, sometimes reaching a depth of 12 to 14 meters, and is a transformation product of the trachytic rocks of the nearby mountains. This transformation is favored by the continuous high temperature and frequent heavy rains during the rainy seasons. Thus the ferric silicates primarily resulting have been further split into ferric hydrate and silicic acid, and finally the brown ferric hydrate has been transformed into red ferric oxid.[1] This characteristic process explains the frequent occurrence in tropical countries of soils of intense red color. The color appears here in such purity and intensity because the formation of humus is almost entirely prevented. The microbes that take part in the production of humus often consume it as quickly as it is formed in a warm climate.

Analyses of a typical soil of this character from the orchard hill near the station by the chemist gave the following results:

Composition of the red clay soils of the Porto Rico Experiment Station.

	Surface soil.	Subsoil.
	Per cent.	*Per cent.*
Insoluble residue	71.34	66.50
Volatile matter	11.55	11.59
Ferric oxid and alumina	16.94	21.70
Lime	.14	.04
Magnesia	.21	.19
Potash	.11	.13
Phosphoric acid	.11	.16
Total	100.40	100.31
Nitrogen	.20	.11
Moisture	5.65	5.39

These figures show that the lime content [2] is rather low, considerably less than the magnesia content. Liming would be of triple benefit, it would improve the lime-magnesia ratio, it would neutralize the acidity, and it would improve the physical condition of the clay. Further, a soil containing calcium carbonate is more favorable to the growth of Azotobacter.

Neither the "sick" nor the healthy soil of the college plantation or the experiment station contained ferrous oxid. Nematodes were searched for in vain in the earth adhering to the roots of sick trees.[3]

[1] Further changes of this "terra rossa" into laterite have taken place only occasionally in this region of Porto Rico.

[2] In regard to the minimum lime requirement of soils, Hilgard (Soils, New York and London, 1910, p. 369) says: "While in sandy soils lime growth may follow the presence of only 0.10 per cent of lime, in heavy clay soils not less than about 0.6 per cent should be present to bring about the same result." Apart from the most extreme cases we may safely augur favorably of any soil containing as much lime as potash.

[3] Semiparasitic nematodes capable of doing some damage are, e. g., the species Cephalobus, Rhabditis, Plectus (Marcinowski, Arb. K. Biol. Anst. Land u. Forstw., 7 (1909), No. 1, pp. 1-192).

A previous examination by the writer had shown, among other things, that in the surface soil *Bacillus mycoides* dominated, then followed *B. proteus*, *B. subtilis*, and *B. fluorescens liquefaciens*. Denitrification was about equally small in "sick" and in healthy surface soil, while desulphurication and butyric fermentations were observed to be more energetic in the "sick" soil. Later determinations showed that at a depth of 12 to 13 inches the butyric bacillus was almost the only microbe present. At a depth of 2 to 3 inches, as well as at 12 inches, Azotobacter was absent. Of protozoa, only *Colpoda cucullus* was present.

Notwithstanding the fact that parasites have not been discovered in the soil and as the increase of butyric bacteria alone could hardly account for the failure of the tree growth, some experiments were made with soil disinfectants. Six sick coffee trees were removed, and the soil in which they were grown treated on April 9, 1910, as follows: (1) 200 grams calcium chlorid; (2) 500 grams calcium chlorid; (3) 200 grams potassium permanganate; (4) untreated; (5) 100 grams commercial formalin; and (6) untreated.

Seedling trees 4 years old were set three weeks later. In April, 1911, trees 1, 2, and 4 were found to be in vigorous growth, while trees 3, 5, and 6 were much less vigorous. In August, 1912, however, all trees, untreated as well as treated, exhibited a more or less sickly appearance. The growth in height had nearly doubled, but there were from 2 to 5 dead branches and 5 to 11 live branches on each of the plants. A number of leaves were attacked by fungi and the leaf miner. Evidently there had been no benefit from the disinfecting compounds, indicating that the trouble was not due to root parasites.

It was probably due to disturbed nutrition, since changes taking place before the dying of the leaves recall similar cases; the chlorophyll disappeared gradually from between the veins and around the margin of the leaves, while near the veins, and especially along the larger ribs the deep green color was still retained for a certain time. This phenomenon, resembling the mosaic disease of tobacco, was observed to be especially marked on leaves of coffee trees at the Aurelia plantation. It was also observed by Harter [1] in cases of malnutrition of cabbage and spinach along the Atlantic coast, where the soils were acid and the roots small and stubby, with few or no laterals. With the sick coffee trees a large portion of the root was found to be dead when a sick tree was removed. It must be recognized that the leaves of weak coffee trees more readily become the prey of parasitic fungi than the leaves of vigorous trees.

At the Aurelia plantation it had been observed that fertilizing did not essentially improve the trees. The fertilized sick trees were much less vigorous than the unfertilized healthy trees. Special care should

[1] Virginia Truck Sta. Bul. 1.

be taken in the selection of fertilizer compounds for acid soils. Such compounds as would increase the acidity of the soil have to be carefully avoided, since the benefit that might be derived from fertilizing is entirely offset by the increased acidity of the soil; thus superphosphate should be replaced by basic slag, potassium chlorid by wood ashes or potassium carbonate, and ammonium sulphate by sodium nitrate. The acidity of soils is considerably increased by such salts as potassium chlorid and ammonium sulphate, because potassium and ammonium are easily absorbed and the acids of the salts are set free. Thus a soluble acidity (so to speak) is created, which is worse than the original insoluble acidity. The failure of fertilizer on the Aurelia plantation may find an explanation in the fact that superphosphate and potassium chlorid were chiefly used.

It is of special interest to observe that while in the college plantation as well as in the Aurelia plantation the sick coffee trees occur on the hilltop, the healthy trees grow on the slopes of the hills. On the same hilltop orange trees and various shade trees, as *Inga vera* and *Roystonea borinquena*, grow very well next to the sick coffee trees. These differences seem to find an explanation in the varying powers of the tree species to develop surface roots. The orange tree develops many more surface roots than the coffee tree, and this is an important matter in such a stiff clay where the aeration is so imperfect. The coffee trees growing on the steep slopes naturally have some of their roots near the surface; the steeper the slope the more favorable is the situation for the roots.

There is, however, a second cause to be considered. In the dry season, which may last from three to four months, these stiff clay soils become hard and show numerous cracks, whereby the smaller roots must be injured. This drying out takes place more on the flat ground of the hilltops than on the slopes, which receive some of the underground moisture.

There exists a third reason for the better growth on the slopes, namely, fallen leaves from the higher lands are washed down the slopes by heavy rains, and, decaying there, furnish mineral nutrients and some ammonium carbonate from the decomposing protein, which not only acts as manure but also helps somewhat to diminish the acidity of the soil. In such naturally fertile bottom lands between the hills the roots need not spread so far in search of mineral food as in the soil not thus situated.

Sick coffee trees also occupy certain areas of the experiment station a mile south of the college plantation. Here only the lower half of the hill slopes bears vigorous trees, on the upper half the trees of the same age are small and sick. The tests for Azotobacter in this soil to depths of 3 and 12 inches showed its complete absence, as with the soil of the college plantation. The flasks stood for eight days at 24

to 33° C., but no trace of a film or of the characteristic diplococci was found in the culture solution, while butyric bacilli were numerous. Of protozoa, only *Colpoda cucullus* was present in the 3-inch soil layer. Determinations of the soil acidity to a depth of 3 inches from 10 different spots showed that in general the acidity decreases toward the bottom of the valley. One kilogram of soil from near the bottom required 0.4 to 0.6 gram sodium hydroxid for neutralization, while farther up 0.7 to 1 gram was required. Although in most cases increased soil acidity is generally correlated with poor development of the coffee tree, yet there are exceptions, and fine trees are growing in soil of the higher acidity. In these cases the trees had either received fertilizer or they may have been naturally fertilized by leaves washed from above.

To test butyric fermentation, 2 grams of each of samples of "sick" and healthy soils taken at depths of 3 and 12 inches were placed with glucose culture solution in the apparatus mentioned previously (p. 11). After 5 days at 24 to 33° C. the results were as follows:

Butyric fermentation of sick and healthy soils.

Depth of sample.	Gas evolution.		Remarks.
	"Sick" soil.	Healthy soil.	
Inches.	*Cc.*	*Cc.*	
3	15.1	7.0	Methyl orange bleached.
12	13.2	13.9	Not bleached.

Hence, while there was no essential difference in butyric fermentation in the "sick" and healthy soils taken at a depth of 12 inches, there was considerably more butyric fermentation in the "sick" soil than in the healthy soil in the 3-inch samples. The roots of coffee trees penetrate to a rather limited extent to a depth of 12 inches in such a heavy clay soil, but mostly occupy the upper layers of the soil.

Several circumstances which have some significance in this connection may be mentioned. In the vicinity of the plantation on the upper hill slope there is a dense grove of gigantic trees of pomarosa and mango, while on the lower slope royal palm, guava, moca, and bucar are more evenly distributed. There are also some large stumps of pomarosa trees [1] with their decaying roots on the upper slopes, while on the lower slopes few and much smaller stumps are distributed through the plantation. On the opposite slope very

[1] The pomarosa root is rather hard and contains much starch and tannin in the bark but no volatile aromatic compounds like the roots of the orange tree, which, on the other hand, do not give a reaction for tannin with ferrous sulphate. The writer was assured by a planter that it is impossible to grow a tobacco plant on a spot from which a pomarosa tree has been removed.

unlike conditions prevail, with fine trees on the lower slope, notwithstanding the very stiff soil, and poor trees above.

The plantation was fertilized twice annually with 15 pounds stable manure per tree with the addition of half a pound of mineral fertilizer. The effect was more striking with the well-developed than with the sick trees, although the alkalinity of the stable manure doubtless exerted a beneficial effect in diminishing the acidity of the soil.

In reviewing the singular distribution of sick and healthy coffee trees in the immediate vicinity of each other, a number of circumstances can be recognized which contribute to cause these remarkable results on such a stiff clay soil, viz:

(1) The coffee trees do not develop an extensive system of surface roots.

(2) The water content during the dry season in upper and lower strata of the same stiff soil is different. Thus the slopes of hills are more moist in the dry season than the tops, and for this reason coffee trees do better on the slopes than on the hilltop.

(3) The sides of the hills receive the washing from the top and upper parts of the slopes and naturally are better supplied with plant food.

(4) The degree of soil acidity and the amount of butyric fermentation differs in different parts of the plantation. This is due to some extent to decaying roots and stumps of felled trees which are rich in starch.

This soil of the college plantation and the experiment station can hardly be called "tired." It suffers, however, from acidity and stiffness. There can hardly be any doubt that after heavy liming and judicious fertilizing it will give good results. It might be best to plant the hilltops with orange trees and only the hill slopes with coffee trees.

SOIL FROM BAYAMON.

A short distance from Bayamon to the west and southwest extends a typical acid soil upon which pineapples and citrus fruits are extensively cultivated with very satisfactory results. In the surface soil sand predominates over an acid clay much more than in the subsoil, which has the character of a loam. The surface soil is somewhat dark, due to a small percentage of humus produced by the use of organic manures, but the subsoil is of a red color. Leguminous plants develop satisfactorily in this soil and show nodules on the roots, even when they penetrate into the more acid subsoil. A determination of the degree of acidity by digestion with 1 per cent sodium acetate, as described on page 7, showed that 1 kilogram of surface soil required 0.6919 gram NaOH for neutralization while for 1 kilogram of subsoil 1.5913 grams was necessary.

As to the butyric fermentation produced by 2 grams of soil at 24 to 30° C. the amount of gas ($CO_2 + H_2$) developed in 30 hours was 13.7 cubic centimeters from the surface soil and 14.6 cubic centimeters from the subsoil, hence the butyric ferment was present in great abundance in the acid soil. Other soils showed such an evolution of gas after 4 or 5 days.

Denitrifying organisms were not found either in the surface soil or in the subsoil. No trace of nitrogen gas was developed in 6 days at 24 to 30° C. when 2 grams of soil was tested in the manner above mentioned.

The test flask examined after standing 5 days at 24 to 30° C. showed Azotobacter to be present both in the surface soil and the subsoil.

Of protozoa, *Colpoda cucullus* was the leading form of Ciliata. Some amœbæ were also recognized, and in the surface soil another infusorium, probably *Colpidium colpoda,* was found. Very few Flagellata were found.

A chemical analysis of this soil had not yet been made, but analyses of various other acid soils of nearby plantations, in the vicinity of San Juan, were available, and in those the content of mineral nutrients was found to be very small. In two cases the potash content was only 0.01 per cent, and in four cases the phosphoric acid amounted to only 0.01 to 0.02 per cent. It is of interest to note also that while in the acid soils in the western part of the island magnesia exceeds lime, those in the vicinity of San Juan contain an excess of lime over magnesia, the latter being found only in traces in three cases. Since the lime content of these soils amounts to only 0.1 to 0.3 per cent, liming with magnesian limestone would be advisable. On the other hand, liming of acid soils in the western part of the island in the vicinity of Mayaguez should be done solely with lime from non-magnesian limestone.

SOILS FROM PONCE.

The hilly country north of Ponce shows extensive tracts of a red acid loam soil upon which coffee is successfully cultivated. Samples of surface and subsoil from the Hacienda Semil were examined for acidity in the manner previously described, and it was found that 1 kilogram of surface soil required for neutralization 0.3954 gram sodium hydroxid, while the subsoil required 1.3838 grams. Hence there is an unusual difference between the acidity of surface soil and subsoil.

SOILS FROM CAGUAS.

The soils in the vicinity of Caguas, in the districts of Rio Grande and Santa Catalina, vary from sandy to loamy in character and show a marked acidity. The amount of sodium hydroxid necessary to neutralize 1 kilogram of soil from the Rio Grande district, taken at

a depth of 10 inches, was found to vary from 0.25 to 0.74 gram. The soil from Santa Catalina required 0.6 gram per kilogram.[1] In the usual test for Azotobacter only the last-mentioned soil yielded after four days a well-developed film of this bacterium, while the soils from the Rio Grande district developed butyric bacilli only. The test was repeated by replacing the glucose of the nitrogen-free culture solution by sodium acetate. After two days the soil from Santa Catalina showed a film of Azotobacter, the infusorium *Colpoda cucullus* in large numbers, small Flagellata and amœbæ, and also threads of what seemed to be a Cladothrix. The flasks, however, containing soils from the Rio Grande district did not develop a trace of Azotobacter. Sodium acetate is not a suitable nutrient for the butyric bacillus, and hence it did not develop here as it did in sugar solution, and since no bacteria developed there was also no development of protozoa, which require a bacterial growth as food. The same soils showed marked development of protozoa as soon as the glucose solution was applied.

In tests for the butyric ferment 2 grams of these soils treated in the manner above described yielded very different amounts of gas in the same time, as the following table shows:

Evolution of gas by butyric bacteria in soils.

Soil sample.	Gas developed in—	
	2 days.	3 days.
Rio Grande:	*Cc.*	*Cc.*
No. 1	1.2	2.9
No. 2	1.1	2.5
No. 5	3.4	8.4
Santa Catalina	5.1	13.8

These differences are perhaps due to difference in manuring, as it is probable that the soil of Santa Catalina has been manured with organic fertilizers for years, while those of Rio Grande have not been so treated.

Denitrifying organisms were present in all six samples obtained. Free nitrogen gas commenced to develop on the third day at 24 to 30° C., when the soils were tested in the manner already described. In five days from 7 to 10 cubic centimeters of nitrogen developed in the different samples. These denitrifying organisms can hardly cause any loss of nitrogen by acting on nitrates of the manure, since in acid media they do not reduce nitrates to free nitrogen. When the secondary potassium phosphate of a weak alkaline reaction in the above-mentioned solution for testing for denitrifying organisms was replaced by monopotassium phosphate, which is of strong acid reaction, no development of nitrogen gas took place.

[1] After liming these soils the yield of tobacco was very much increased and the quality improved.

SOILS FROM RIO PIEDRAS.

Two samples were examined as to acidity. One sample came from the hilly country in the vicinity and was a dark-brown clay from the surface and the other came from the bottom land at a depth of 12 inches and was a light-brown clay soil. The acidity tests showed that the subsoil from the bottom land is rather strongly acid, while the surface soil from the hills is only moderately so.

NOTE ON ALKALINE SOILS.

The term "alkaline soils" is here restricted to soils which showed a more or less strong alkaline reaction with litmus, due to sodium or potassium carbonate. Such soils exist especially in countries with a very dry climate, where these salts, formed by disintegration of the rocky particles of the soil, can not be removed by leaching. Comparatively dry regions exist on the south side of Porto Rico, where the central mountain range prevents the clouds from the north crossing to the southern side. Such a district is that of Santa Rita, three samples of alkaline soils from which were examined. No. 1 came from Fraternidad, No. 2 from Boneli, and No. 3 from Cinco Hermanos. The three soils were very tenacious gray clays. After digesting 50 grams of the fine earth with 200 grams of water, with frequent shaking, for 24 hours the mixture was filtered. Of this alkaline filtrate, 100 cubic centimeters was neutralized by titrating with twentieth-normal hydrochloric acid. The following results were obtained:

Relative alkalinity of some Porto Rican soils.

Sample.	Twentieth-normal HCl required to neutralize 100 cc. of filtrate.	HCl required to neutralize 1 kg. of soil.
	Cc.	*Gm.*
No. 1	6.5	0.4743
No. 2	8.2	.5986
No. 3	28.0	2.0440

Soil No. 3, showing the strongest alkalinity, yielded a colloidal clay solution which coagulated when acids or neutral salts were added. When 10 grams of soil was treated with 40 cubic centimeters of water and then 2 grams of ammonium chlorid added, a clear filtrate was obtained which, after evaporation and removal of the excess of ammonium chlorid by ignition, yielded a residue consisting of sodium chlorid. Hence the alkalinity of the soil is caused by sodium carbonate.

It was observed that the butyric bacillus occurred to a large extent in the weakly alkaline soils, Nos. 1 and 2, and to a moderate extent in the strongly alkaline soil, No. 3, as shown by the comparison of the intensity of the butyric fermentation and microscopic examination.

In the tests for Azotobacter the weakly alkaline soils developed after two days fragments of a film in which the bacteria resembled *Azotobacter thermophilum* rather than the common *A. chroococcum*. The stronger alkaline soil, No. 3, did not yield any Azotobacter film.

The protozoa, *Colpoda cucullus*, and a smaller species were observed in the weakly alkaline soils, Nos. 1 and 2, but in the strongly alkaline soil no trace of any protozoa was present.

OBSERVATIONS ON NITROGEN GATHERERS.

The observations with special reference to the behavior of the two nitrogen gatherers, *Bacillus butyricus* and Azotobacter, in Porto Rico soils may be summed up as follows:

(1) Soils of Porto Rico are frequently acid. This acidity is due to an acid clay, and can be best determined by treatment with sodium or potassium acetate and titration of the acetic acid set free.

(2) The butyric ferment was found in alkaline soils and in all acid soils tested, even to a depth of 18 inches in a very stiff acid clay soil.

(3) A measure for the relative content of the butyric ferment in soils can be obtained by comparing the amount of gas developed with different soils when placed with nitrogen-free glucose culture solution in a suitable apparatus.

(4) Azotobacter was found not only in moderately alkaline soils, but also in soils of considerable acidity. When, however, these acid soils consisted of a very stiff clay with deficient aeration it was absent.

(5) Liming of acid soils had a very favorable effect on the growth of Azotobacter. The limed soils yielded a luxuriant film of Azotobacter much sooner than the unlimed check plat did. This increase of Azotobacter is in accord with the observations that the nitrogen-fixing power of soils is increased by liming.[1]

[1] Cf. Brown, Iowa Sta. Research Bul. 5.

It was observed that the butyric bacillus occurred to a large extent in the weakly alkaline soils, Nos. 1 and 2, and to a moderate extent in the strongly alkaline soil No. 3, as shown by the comparison of the intensity of the butyric fermentation and microscopic examination.

In the case of the Azotobacter the weakly alkaline soils developed after two days' incubation a film in which the bacteria resembled Azotobacter, [illegible]. The strongly alkaline soil No. 3 did not yield the Azotobacter film.

The protozoa, Colpoda cucullus, and a smaller species were observed in the weakly alkaline soils, Nos. 1 and 2, but in the strongly alkaline soil no trace of any protozoa was present.

CONCLUSIONS ON NITROGEN GATHERERS.

The observations with special reference to the behavior of the two nitrogen gatherers, Bacillus butyricus and Azotobacter, in Porto Rico soils may be summed up as follows:

(1) Soils of Porto Rico are frequently acid. This acidity is due to [illegible], and [illegible] potassium [illegible] of the [illegible].

(2) The butyric bacillus was found in alkaline soils and in all acid soils tested, even to a depth of 18 inches in a very stiff and clay soil.

(3) A measure for the relative amount of the butyric bacillus in a soil can be obtained by comparing the amount of gas developed with different soils when placed with nitrogen-free glucose culture solution in suitable apparatus.

(4) Azotobacter was found not only in moderately alkaline soils, but even in soils of considerable acidity. When, however, the acid soil consisted of a very stiff clay with deficient aeration it was absent.

(5) Lime and [illegible] soils had a very favorable effect on the growth of Azotobacter. The limed soils yielded a dominant film of Azotobacter much sooner than the unlimed check plot did. The increase of Azotobacter is in accord with the observations that the nitrogen-fixing power of soils is increased by liming.[a]

[a] [illegible]